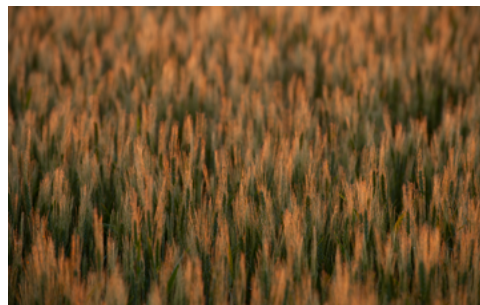




Chapter 31: Using Molecular Biology for Management and Genetic Enhancements



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The purpose of this chapter is to describe how molecular biology, molecular enhancements, and integrated crop production research can lead to genetic improvements and the development of management systems that fully utilize the genetic capacity of wheat varieties.

Rules of Thumb for Using Molecular Biology to Increase Profitability

- Molecular biology provides information that speeds up crop breeding by approximately 50%.
- Unlike corn, soybean, and rice, the sequencing of the wheat genome continues. When the wheat genome is sequenced (estimated 5 years), the ability to enhance both genetics and management practices should be improved.
- Molecular biology provides information that can be used to better understand how genes, climate, and management interact.

Introduction

Researchers are often asked how our experiments with genes and DNA in the laboratory can possibly benefit farmers and their crops out in the field. Sometimes, research scientists seem just too far removed from the reality of what happens on the land. Can these molecular approaches really lead to benefits for growers and producers? The short answer to that is yes. Information produced by molecular biology can be used to:

- Improve our understanding on how wheat grows and develops.
- Develop information that can lead to improved Best Management Programs.
- Speed up and increase the efficiency of wheat breeding programs.

Our ancestors started manipulating genes in wheat around 12,000 years ago. These early farmers had the same goals as we do, and in many ways used similar approaches to improve their crops. The wild relatives of our crop plants had many undesirable qualities that made early farming much harder than it is today. In 1884, Alfonse De Candolle wrote in *Origin of Cultivated Plants* that:

a cultivated species varies chiefly in those parts for which it is cultivated ... We may expect, therefore, to find the fruit of a wild fruit tree small and of a doubtfully agreeable flavour, the grain of a cereal in its wild state small, the tubercles of a wild potato small, the leaves of indigenous tobacco narrow ... (13-14)

The transformation from wild to domesticated varieties is called the *Domestication Syndrome*. In this process, spontaneous mutations occur in wild populations and these mutant individuals are selected for use by humans for their more desirable traits. Interestingly, the traits selected for “under” domestication would often be detrimental to the crop in the wild. As a consequence, fully domesticated crops may not survive in the wild without human intervention.

Wheat provides an excellent example of this. The ears of wheat are separated from the stem that bears them by a structure called the *rachis*. Wild forms of wheat need to disperse seeds effectively, so they have easily shattered ears with brittle rachises. When the wheat seeds mature, the rachis shatters and the seeds penetrate surface litter embedding into ground cracks. This is an important mechanism for effective seed dispersal. The problem with this is that when the seeds fall they also become difficult for humans to gather. Wild forms of wheat, such as Wild Emmer, have a brittle rachis, therefore making harvesting time consuming and inefficient.

During early wheat domestication, farmers selected for a rare single gene mutation (*br* – brittle rachis) that prevents shattering (Dubcovsky and Dvorak 2007). This mutation is lethal in the wild (because the seeds fail to drop), but conveniently concentrates the seeds for human gatherers. All domesticated forms of wheat have this mutation.

Wild wheat also had tough glumes, making threshing difficult. A genetic mutation converting hulled wheat into free-threshing wheat was selected for, and is present in Durum and Bread Wheat, but not Emmer.

The main gene that is responsible for this free-threshing habit is called *Tg* (tenacious glume). Another gene that also produces free-threshing wheat is simply called *Q*. *Q* is a transcription factor. Transcription factors are proteins that turn other genes on or off. Molecular biology has shown that most of the key domestication genes in wheat and other cereal species are transcription factors (Table 31.1). Understanding transcription factor genes is important because mutation in a single factor can turn a whole process on or off.

Table 31.1. Selected genes and their roles in cereals.

| Gene | Crop species | Type of gene | Role |
|---------------|--------------|------------------------------|------------------|
| <i>Q</i> | Wheat | AP2 Transcription factor | Free-threshing |
| <i>Rht-B1</i> | Wheat | GRAS Transcription factor | Semi-dwarf plant |
| <i>Tb1</i> | Maize | TCP Transcription factor | Lateral branches |
| <i>TGA</i> | Maize | SBP Transcription factor | Glume size |
| <i>SH4</i> | Rice | MYB Transcription factor | Grain shattering |
| <i>qSH1</i> | Rice | BELL HD Transcription factor | Grain shattering |

The family history of wheat

Molecular biology can speed up breeding programs. To understand how these new molecular methods can do this, first we need to consider wheat itself. First, wheat is different in several important respects from corn, rice and barley. Bread wheat is the result of multiple crosses between goat grass (the “grandmother” of wheat—**gene set AA**) and wild wheat (the “grandfather” of wheat—**gene set BB**). The progeny of these crosses enabled a second type of cross between durum (called the “mother” of wheat—**gene set AABB**) and another goat grass (called the “father” of wheat—**gene set DD**) (Fig. 31.1).

The result is that Bread Wheat has three genomes: the A, B, and D genomes. This cross (**gene set AABBDD**) was probably made by ancient farmers living in what we now call Iraq. The resultant cross demonstrated “hybrid vigor,” and outperformed its wild ancestors in yield and environmental adaptation, leading to further cultivation and hybrid improvement. The initial crosses, which were a boon to ancient farmers, also had the unfortunate side effect of creating an extremely complex genetic code for twenty-first century scientists.

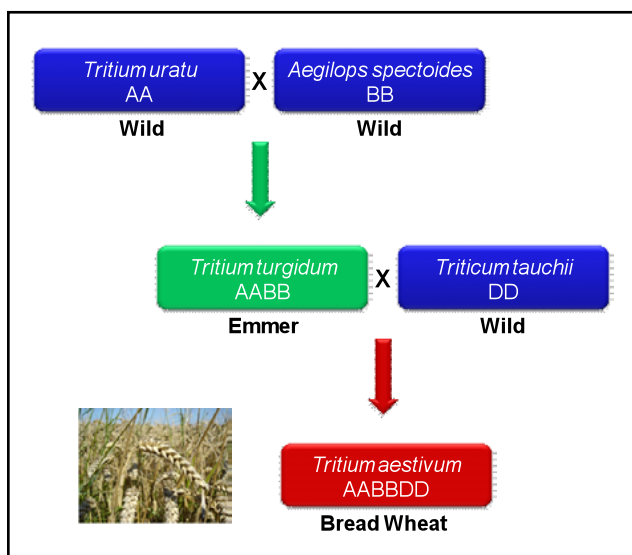


Figure 31.1. A diagram showing the genetic source of bread wheat. (Source: Paul Rushton)

The complete set of genes (a gene is a section of DNA that is responsible for traits) that makes up any living organism is called its **genome**. This can be compared to a large book of blueprints, for instance, or the code that makes up a computer program. Wheat has a huge genome that is forty times larger than that of rice, and fifteen times the size of soybean. Because of its genetic background we described previously, wheat potentially has three genes for each trait. This complicates matters considerably. For example, if we want to produce an improved wheat variety by eliminating a gene with negative properties, we are faced with the possibility that we will have to actually eliminate three genes because each of the three genomes has a copy of this gene. Eliminating only one gene is likely to have no effect because there will still be two of these genes left that can carry out the job that this gene performs.

The sequence of the wheat genome

To effectively use molecular biology for genetic improvement, we need the genetic code. Given that wheat contains three genomes, making sense of the genetic code is like trying to assemble three jigsaw puzzles that have been mixed in the same box. It was therefore a surprise to wheat scientists when it was recently announced that the wheat genome had been sequenced. This accomplishment, although immensely important, was not quite what it seemed. Unfortunately, what the group of scientists had produced was not a genome sequence in a form that scientists and breeders can easily use.

A simple jigsaw puzzle example again explains what happened. The scientists had chopped the wheat genome up into small chunks and sequenced all of the chunks—just like making all of the pieces of a jigsaw puzzle, without putting the puzzle together. Without putting the pieces of the jigsaw puzzle together, you cannot see the complete picture. Exactly the same is true of the sequence that was generated from wheat in the UK. The small pieces of DNA sequence now need to be put together (assembled) before we can use them.

The International Wheat Genome Sequence Consortium, a more than 200-member organization of growers, breeders and scientists not affiliated with the U.K. project, issued a press release in Washington, D.C. on August 30, 2010 <http://www.wheatgenome.org/News-and-Reports/News/Significant-Work-Still-Needed-to-Really-Crack-Wheat-s-Genetic-Code>. They echoed what the UK funding body had said, namely that “significant work remains to be done to achieve a complete genome sequence” <http://www.bbsrc.ac.uk/news/archive/2010/2010-archive-index.aspx>.

In contrast, the International Wheat Genome Sequence Consortium is attempting to produce the complete assembled wheat genome “in the next five years.” This seems a reasonable target. Some believe that the develop of the wheat genome will be “the most significant breakthrough in wheat production in 10,000 years.” Current wheat growers live in exciting times.

Why will the wheat genome sequence be such a big breakthrough?

Our forefathers improved wheat in an untargeted way. They observed mutants and if this resulted in an improvement, they selected from those plants. It was completely dependent on the occurrence of natural mutations, which is a slow process. By contrast, modern molecular approaches are rapid and highly targeted.

We take a specific gene or genes and alter it. We then monitor the effect to see if there is an improvement in the wheat cultivar in some important trait such as yield or resistance to disease. To do this effectively, however, we need all of the genes in wheat so that we know what to manipulate. We can't modify something if we don't know of its existence. The genome sequence provides the blue print for this approach.

When a plant is affected by drought or water stress, are there management practices producers can implement to help reduce the yield loss? Probably. Research on corn indicates that corn plants under water-deficit stress in summit landscape positions are more susceptible to disease and nutrient stress than non-water-deficit corn in footslope positions (unpublished data, Clay et al.). This can be compared to a human's immune system; if you stress a person by withholding water, nutrients, or sleep, the person's immune system will be lowered. It seems to be the same with plants.

Because not all fields have consistent nutrient or water availability, applying a field-wide fungicide can be wasteful, as some areas just don't need it. Molecular biology can lead to in-field tests that will allow producers to assess the activity of specific genes. This information can be used to improve management decisions. At the present time, producers and consultants can make assumptions regarding what management options are needed, but until we understand what is really happening inside the plant, they are just “best-guesses.”

What are “molecular approaches?”

Molecular techniques can be integrated into traditional breeding and agronomic production approaches. When linked with breeding, molecular biology techniques speed up the cultivar selection process. Molecular approaches can also provide critical field production information needed to take full advantage of the genetic potential of crops. For example, by using molecular biology, the impacts of seeding density, fertilizer rates, and heat stress on the up-and-down regulation of specific genes can be assessed. The bottom line is that molecular biology enhances traditional testing approaches. Molecular tools that are routinely used in breeding and crop production research are molecular markers and micro-arrays. The

development of transgenic corn and soybeans relied on molecular approaches.

Molecular markers

A breeder crosses one wheat variety with another, getting 500 seeds that potentially contain the trait being breed for. Before Marker Assisted Selection (MAS), the breeder would have to grow out all 500 seeds and assay them for the desired trait, sometimes to full maturity, depending upon which trait was being sought. This method uses valuable greenhouse or field space, labor, and resources. Using MAS, breeders can germinate the seeds, take a small tissue sample, and save the seedlings that have the marker for the desired trait.

Perhaps only ten of the 500 seeds contain the trait, but breeders will now only have to grow out those ten plants knowing they contain the desired trait. Markers are unique, short strings of DNA located near a gene of interest. Small genetic differences in the DNA sequence of traits can be responsible for one plant being resistant to a disease and another not being resistant. Using MAS, the time required to bring a new trait to the public is reduced by 50%. In wheat, approximately 6,000 molecular markers have been discovered. These markers function as an additional set of “index tabs” in the wheat set of blueprints.

Microarray technology assesses plant responses to stress

Plants respond to soil, climate, and pest stress by changing the genes that are expressed. Microarray (or chip) technology allows us to pinpoint which genes have been affected by stress treatments by comparing the gene expression of a control plant to the gene expression of a test (or treated) plant. Wheat chips have been used to explore gene expression during pathogen infection, environmental stress, and plant development. In corn we have used microarray analysis to assess the influence of plant density and weed competition on gene expression. Understanding what is happening in the plant under stressful conditions will lead to better decision making regarding planting populations, choice of variety, fungicide and fertilizer applications, and other management decisions.

Some routes to improved wheat varieties

Traits that wheat breeders are specifically interested in include: vernalization and photoperiod response, plant architecture, grain quality, pest resistance, and tolerance to abiotic stresses. **Vernalization** and **photoperiod responses** are of interest because they influence the wheat flowering time. Increasing the length of time of grain filling may lead to higher yields.

Plant architecture is important because it impacts the ability of the plant to withstand lodging. As stand density and use of fertilizer increased, lodging became a critical problem. Approaches to solve this problem are breeding shorter plants and delaying N fertilizer applications (Chapter 11). One of most significant contribution of the “green revolution” is the reduction of wheat plant height. Two *reduced height* (*Rht*) (Table 31.1) genes are now found in most modern semi-dwarf wheat cultivars. The manipulation of these genes significantly reduced wheat plant heights to 80 to 90 cm and improved wheat resistance to lodging.



Figure 31.2. Lodging in wheat has been greatly improved by selective breeding centered on the two *Rht* genes. (Photo courtesy of <http://faculty.uca.edu/johnc/greenrev3390.htm>)

Research has been conducted to understand how genetics, management, and climate interact to impact **grain quality** (protein composition, baking, and mixing characteristics). Progress has been made in understanding the genetic components of wheat grain quality in two aspects: grain hardness and grain protein content. Genes controlling hardness of wheat have been discovered (PinA and PinB genes) (Hogg et al. 2004). Varieties with specific mutations (sequences) in these genes are hard textured, while other varieties have sequences contributing to the soft wheat type.

Genes controlling the protein content of wheat have a direct effect on the bread-making quality of the grain produced. Several of these genes that effect protein content have been discovered in wheat and have been manipulated in modern-day wheat, with more improvements to come. <http://deltafarmpress.com/promise-better-wheat-varieties>

Disease resistance research has focused on rotational effects as well as better understanding seedling and adult plant resistance. Seedling resistance is race-specific, whereas adult plant resistance is broad-spectrum. Adult plant resistance is more durable, although at lower levels compared to seedling resistance. More recently, progress has also made in understanding wheat plant susceptibility to rusts, powdery mildew, tan spot, and plant resistance to Hessian fly and greenbugs. Improving disease resistance is one of the areas where molecular biology holds most promise for breeders and growers.

Wheat production is facing numerous challenges from drought, excess water, heat, salinity, and other soil-derived toxicities. Complex traits such as drought and heat-stress tolerance have started to reveal themselves through the use of molecular tools. It will take hard work and time to understand traits such as these so that we may use them to our advantage. By better understanding how the plant responds to stress, we can develop more effective management practices.



Figure 31.3. Fusarium head blight and rust are two diseases that researchers are battling through molecular means. (Photo courtesy of Mary Burrows, Montana State University, Bugwood.org)

In the future by linking our breeding, crop production, and molecular biology programs, we will be more effective at producing resilient tools that can respond to climate variability. Compared to rice, maize and soybean, a bottleneck limiting wheat research is the lack of a complete set of “blueprints.”

Currently, labs in 16 countries within the International Wheat Genome Sequencing Consortium (IWGSC) are engaged in decoding this immense book of blueprints. This knowledge will increase our capacity, efficiency, and dimensions in dealing with complex traits, such as drought and heat tolerance. Successful decoding of these blueprints will unlock the bank and pave the way to realize the potential of the sleeping wheat germplasm. As mentioned above, wheat growers in the twenty-first century live in exciting times.

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